AN UPDATE

Guidelines for setting up of a Molecular Laboratory

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ABSTRACT

Molecular laboratories are now an essential part of diagnostics. The combination of sensitivity, specificity and speed has made molecular techniques appealing methods for the diagnosis of many diseases. While the requirements for setting up a molecular lab may vary depending on the spectrum of the tests that will be performed, there are several basic criteria that need to be fulfilled for standardization. Adequate space, appropriate equipment and qualified personnel are required to establish a molecular pathology laboratory. It is essential to take steps to prevent contamination. In this review, the criteria required to establish an optimal molecular pathology laboratory will be reviewed.

INTRODUCTION

Molecular diagnostics is a technique used to analyse biological markers in the genome and proteomeby applying molecular biology to medical testing. It is the detection and/or analysis of bio-molecules (DNA, RNA and Protein*)* for diagnostic and therapeutic purposes. The molecular laboratory is increasingly becoming an integral part of all laboratories. This is not only because of the advent of targeted therapies and personalized medicine in oncology but also as a gold standard diagnostic test in various benign conditions. The technologies that constitute molecular diagnostics include first-generation amplification, next-generation DNA sequencing, deoxyribonucleic acid (DNA) probes, fluorescence in situ hybridization (FISH), second-generation biochips next generation signal detection, biosensors, and molecular label[1,2].

Interestingly, the establishment of molecular laboratory methods, the choice of the technique, the sample workflow, the quality control and the genomic alterations to be detected, are always left to the discretion of the laboratory. The purpose of this article is to describe the different steps of the settlement of a molecular genetic platform in a pathology laboratory.

Take the first step, howsoever small

As a first phase, molecular laboratories are established to develop a selection of genes to evaluate a few genomic alterations. In a second phase, the number of studied genes is increased. The challenge to laboratories is to meet the increasing need, using reliable methods and processes to ensure that patients receive a timely and accurate report on which their treatment will be based. On the successful launch and utilization of real-time PCR technologies, lab can further go to Phase 2 analytical instruments like sanger (dye termination) sequencing and/or NGS.

Scope of Applications of Molecular Diagnostics

From the detection, quantitation, genotyping of various infectious agents (Viruses, bacteria, fungi, and parasites) to detection of defective genes and variations in the genome, the scope of molecular genetics is tremendous. Congenital genetic disorders, diagnosis of existing disease or predisposition to any, malignancies, gene defects, expression profiles related to cancer, pharmacogenetics (identification of slow, average, fast metabolizers, non-responders) to optimize drug therapy, paternity, forensic medicine and transplantation, the uses of molecular techniques are far from imagination.

Three basic requirements for setting up a molecular lab

To begin with, the three broad requirements for a molecular lab are:

1) Adequate and well organized space
2) Essential lab equipments

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3) Well trained and competent staff including doctors, lab technicians, post docs.

These three requirements will be discussed in detail.

Workflow for molecular diagnostics:1) Organizing the space

The various steps of molecular diagnostic lab include:1) sample collection 2) DNA/RNA extraction 3) DNA amplification 4) real time analysis 5) gel analysis. The entire workflow is divided into two main phases a) pre -analytical and b) analytical (figure 1).

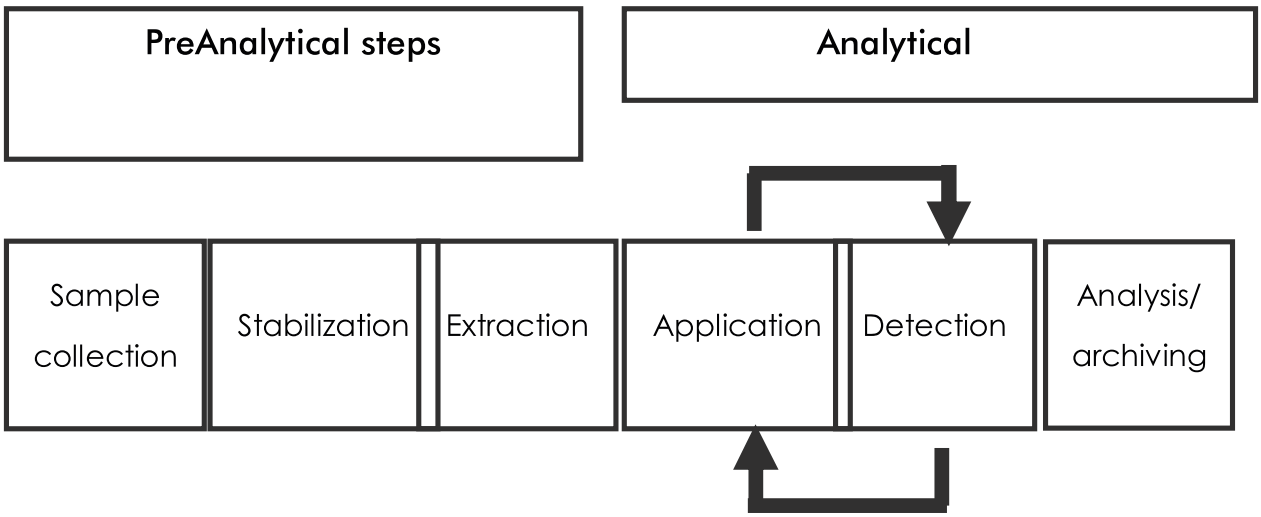

Figure 1: Workflow of a molecular lab

Preanalytical considerations

An important step in molecular testing is a careful consideration of the way in which specimens are obtained and reach the laboratory[3]. Sample receipt and handling follow standard operating procedures. DNA and RNA extraction need to be standardised and should be checked for quality and quantity of output on a regular basis. Internal quality control, regular internal audit of the whole testing process, laboratory accreditation, and continual participation in external quality assessment schemes are prerequisites for delivery of a reliable service. A molecular pathology report should accurately convey the information the clinician needs to treat the patient with sufficient information to allow for correct interpretation of the result.

Ideal physical conditions

The ideal is a 5-roomset up, though in case there are space considerations it can be done in a 4/3-room set up also (Figure 2). The three minimum areas are:

Area 1 – Reagent preparation: it is the room where reagent stocks are prepared and then divided into a certain number of small usable parts (aliquoted), and the reaction mixes are prepared. This room should be free of any biological materials such as DNA/RNA extracts, PCR products.

Area 2 – Specimen/control preparation, PCR set-up:It is where the nucleic acid isolation is performed and the isolated samples are added to the PCR reaction mixes. This room is also called a "low copy" room, as the number of copies has not yet been amplified by PCR. Ideally, it is recommended to perform the steps of nucleic acid isolation and addition of isolated samples to the PCR reaction mixes in separate rooms. Preparation of the PCR reactions in a laminar flow biosafety cabinet ensures that the area remains clean.

Area 3 – Amplification/product detection, plasmid preparation: It is where PCR devices are located and the amplification steps are performed.

Two of these rooms are considered clean rooms in that all tasks and duties before in vitro amplification are performed in these laboratories[4]. These rooms are called preamplification rooms. The third room is considered a dirty room because it is dedicated for in vitro amplification and analysis of the amplified material. This room is called a postamplification room.

The preamplification laboratories should be under positive air pressure, which impedes the entrance of any airborne contaminant into the preamplification

laboratory when the door is opened. In addition, the postamplification laboratory should be under negative air pressure to impede anything from coming out of this laboratory and contaminating the surrounding environment.

Ideal workflow

There should be mechanical barriers between these areas to prevent contamination. The workflow should always be **unidirectional** for all personnel, including cleaning personnel, and specimens. Remove PPE before leaving one area. Avoid or limit reverse direction. Reusable supplies in the reverse direction need to be bleached. Reagents used for amplification should not be exposed to other areas. Size of each area should consider space for equipment and bench space needed for preparation. Other laboratory design considerations include temperature and humidity requirements, exhaust ventilation, water quality, electric outlets, back-up power system and ergonomic assessment.

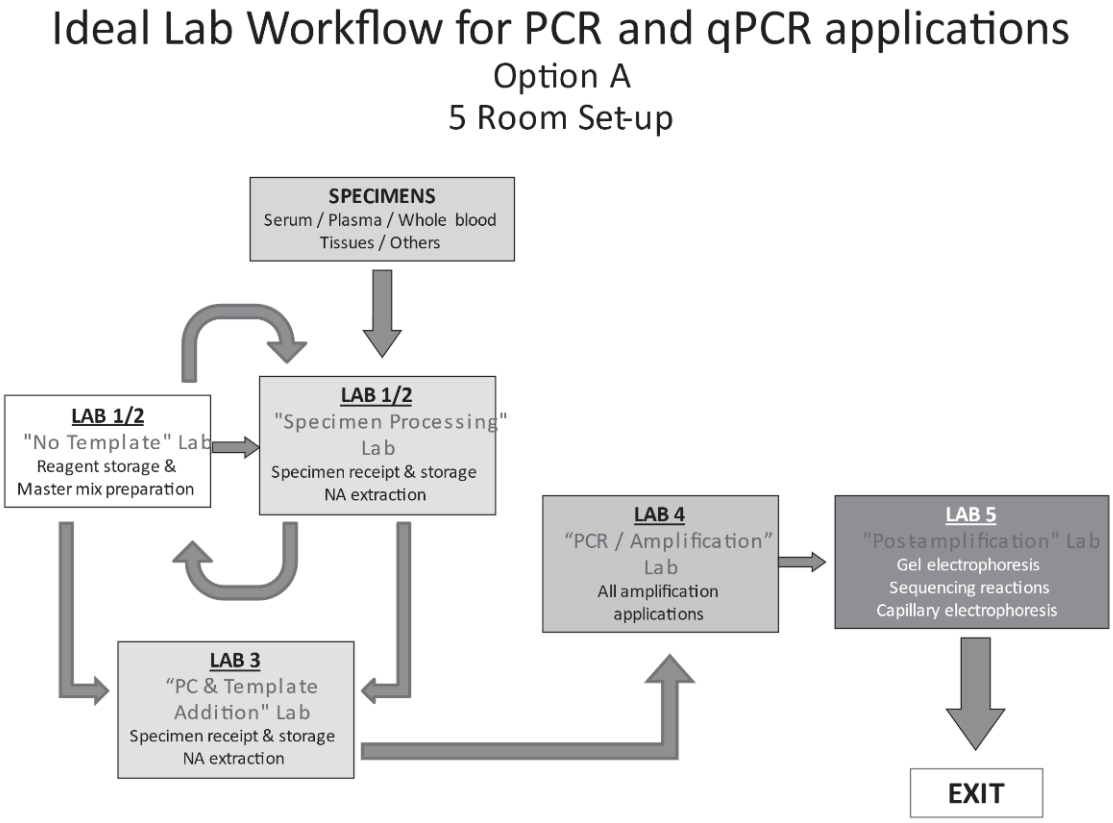

Figure 2:Ideal workflow for molecular lab

2) Equipment required for a molecular lab

PCR labs typically require a variety of equipment, such as centrifuges, vortex mixers, pipettes, fridges and freezers, thermal cyclers and analysis instruments (e.g., electrophoresis systems). Depending on the size of lab and applications, the number of equipment may vary. Since aerosols are always a concern, a laminar flow hood or biosafety cabinetis must. The main equipment required in each area is described in table 1.

Table 1: Ideal lab set-up for PCR and qPCR applications

Area	Equipment
Common Instrument Area	Ice Flaker
	Autoclave – Front loading / manual / digital
	pH Meter – Digital / manual
	Plate Shaker
Specimen Processing	Biosafety cabinet
	Refrigerated Centrifuge
	DNA/RNA extractor
	Mini Spin and vortex
	Dry Heating Block
	Spectrophotometer (Nano Drop / Qubit)
	Dedicated pipette Set
	Chillers (4° C) and Refrigeration System (-20° C) and (-80)
Reagent Preparation	PCR Hood / workstation / clean hood
	Mini Spin and vortex
	Dedicated pipette Set
	Refrigeration System (-20° C)
Amplification / PCR	Thermal Cycler
	Real-Time PCR instrument
	DNA Sequencer
Post - Amplification	Microwave over, Gel Electrophoresis System with Power Packs and Casting Units
	Gel Documentation
	Automated Elisa System

3) Well trained staff

Machines cannot run on their own and can definitely not replace human mind. Experienced and trained doctors, researchers and technicians run the machines. Expertise in the molecular biology field along with the dedication of the staff towards running the lab is one of the most essential requisites for successful running of a lab. All that glitters are not gold; the shining molecular lab often has volumes to tell about the hard work of the involved staff. It is essential for staff to remain updated about the latest techniques of molecular biology.

Quality Management Components

Various aspects of the molecular lab require quality management. These include proper organization, trained personnel, maintenance of documents and records, environment and safety and use of referral laboratories whenever needed[5,6].

Laboratory Practices which ensure quality are use of positive displacement pipettes and disposable filtered pipette tips, avoiding production of aerosols when pipetting, use of sterilized single-use plasticware, use of hairnet and dedicated safety glasses, disposable lab coat/gown, and shoe covers. Use of nuclease free or autoclaved water avoiding multiple freeze thaws which can cause degradation. Always include a blank (no template) control to check for contamination.

Contamination

Introduction of unwanted nucleic acids into specimen which hampers the sensitivity of PCR techniques makes them vulnerable to contamination. Repeated amplification of the same target sequence leads to accumulation of amplification products in the laboratory environment.Build-up of aerosolized amplification products will contaminate laboratory reagents, equipment, and ventilation systems.

The potential sources of contamination include:

1) Cross contamination between specimens
2) Amplification product contamination
3) Laboratory surfaces

4) Ventilation ducts
5) Reagents/supplies
6) Hair, skin, saliva, and clothes of lab personnel.

Decontamination Approaches

Always chose sterile products from manufacturers that can certify that their tips and consumables are free of any of these potential contaminants[7]. Clean the work area & equipment routinely, clean the PCR workstation at the start and end of each work day/run (UV light, 70% ethanol, fresh 10% sodium hypochlorite). Clean the exterior and interior parts of the pipette. Clean the equipment, doorknobs, handle of freezers. Despite being more expensive than normal pipette tips, using filter tips for your PCR set-up will avoid aerosols entering and contaminating your pipette, and avoid aerosols that might already be present in your pipette contaminating your master mix or samples. Adding the sample last is also recommended Aliquoting reagents into smaller containers will increase their shelf life and prevent them from going through too many freeze/thaw rounds[8].

When is a Validation/Verification Study Required?

Whenever a new testing system or a new analyte is introduced in the lab or an analyte previously measured/detected is now measured on an alternate system, a validate is required.

Types of Controls

The types of controls that are used are:

1) Positive amplification controls: it checks whether the experiment is capable of recovering and amplifying the target. It detects failure of DNA extraction or PCR amplification, reagent or equipment issues, Integrity of DNA sample, presence of inhibitory substance. Negative control checks for contamination of PCR experiment with foreign DNA. This includes 2) Negative Control: checks if there is any contamination of the experiment with foreign DNA. 3) No template control (extraction blank): checks if PCR reagents are contaminated. 4) Internal Control. 5) External Control

CONCLUSION

It is necessary to carefully design the molecular diagnostics laboratory to ensure sufficient and adequate space for personnel and equipment and also to minimize the potential problem of specimen contamination. The ideal lab set up is sometimes far from real and the labs may have to adjust in terms of space, staff and budget. It's best to just get started and evolve!

Future directions: The use of molecular laboratory in molecular diagnostics, such as pre-implantation diagnostics or predictive genetic testing, still has technical problems as well as a novel and unclear, social, ethical, and legal implications. The scope of molecular laboratory in molecular medicine could be expanded well beyond current nucleic acid testing which plays an important role in the practice of medicine, public health, pharmaceutical industry, forensics, and biological warfare, and drug discovery. The molecular laboratory marketplace offers a growth opportunity given the interest in utilizing molecular tools to precisely target therapeutics.

Take Home Messages

- ✓ The molecular laboratory has become a growing part of the clinical laboratory.
- ✓ Separate laboratory spaces are needed for reagent preparation, sample preparation, amplification and detection.
- ✓ Unidirectional workflow is a must.
- ✓ Precautions and special laboratory practices must be made to minimize the risk of contamination and decontamination steps should be done.
- ✓ A quality control plan to monitor the quality of testing process and detect errors should be in place.
- ✓ Continuous quality improvement is essential.
- ✓ Accreditation is going to be vital and unavoidable.

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